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Parasitoid Mark-Release-Recapture Techniques—II. Development and Application of a Protein Marking Technique for *Eretmocerus* spp., Parasitoids of *Bemisia argentifolii*

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In this study, we validate and apply techniques for marking and capturing small parasitoids of the silverleaf whitefly, Bemisia argentifolii *Bellows & Perring [=* B. tabaci *(Gennadius), strain B] for mark-release-recapture (MRR) studies. The marker is the purified protein, rabbit immunoglobulin G (IgG), which was applied externally by topical spray or internally by feeding. Marked parasitoids were then assayed using a sandwich enzyme-linked immunosorbent assay (ELISA) for the presence of the protein marker using an antibody specific to rabbit IgG. Virtually all of the externally marked* Eretmocerus *sp. (Ethiopia, M96076) (98.0%) contained enough rabbit IgG to be easily distinguished from unmarked parasitoids, regardless of the amount of protein applied or the post-marking interval. A field MRR study was then conducted to examine the dispersal characteristics of* E. emiratus *Zolnerowich & Rose. Parasitoids marked externally and internally with protein were released on three separate trial dates into the center of a cotton field bordered by cantaloupe and okra. Overall, a total of 1388, 637, and 397 marked and unmarked wasps were captured in suction traps during each trial, respectively with the majority of parasitoids captured between 0600 and 0800 h. Furthermore, even though we released an equal proportion of males to females, our traps consistently contained more males. Our results suggest that there are gender-specific differences in the dispersal behavior of* E. emiratus. *Almost 40% of the captured parasitoids collected during the three release trials were positively identified for the presence of the protein marker. The distribution of the marked parasitoids revealed two distinct patterns. First, almost all of the marked parasitoids recaptured in the cotton plot were in suction traps at or adjacent to the*

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release site. Second, marked parasitoids were recaptured more frequently in distant traps located in the cantaloupe plot than in distant traps located in the cotton and okra plots, thus suggesting that the parasitoids were moving toward the cantaloupe plots.

Keywords: *insect marking, ELISA, mark-release-recapture, dispersal*

INTRODUCTION

It is critical that parasitoids released for pest control occupy their intended target site. However, quantifying parasitoid dispersal is often difficult because of the small size of parasitoids. Generally, parasitoid dispersal is researched by mark-release-recapture (MRR) techniques (Southwood, 1978). MRR involves mass-marking insects with a marker, releasing them into the field and recapturing them at given time and distance intervals after dispersal.

Knowledge of the mobility of released parasitoids is an important factor in determining their efficiency as a biological control agent. For example, applicators of parasitic biological control agents need to know how many, how often, and how far apart to release parasitoids for effective pest management (Simmons, 2000). Unfortunately, for many parasitoid species this information is lacking. A major barrier to tracking parasitoid movement effectively in the field is the lack of reliable and user-friendly methods for marking parasitoids (Hagler & Jackson, 2001). Researchers have used a wide variety of markers for insect dispersal studies (Southwood, 1978; Akey *et al.*, 1991); however, most methods (e.g. paints, dyes, dusts etc.) are not effective for marking small and delicate parasitoids (Hagler & Jackson, 2001). Probably the best markers used to date for monitoring parasitoid dispersal are trace elements such as rubidium, cesium and strontium (Jackson *et al.*, 1988; Jackson & Debolt, 1990; Corbett *et al.*, 1996; many others). Trace elements are environmentally safe and retained in or on many insects including minute parasitoids (Berry *et al.*, 1972; Stimmann, 1974; Jackson *et al.*, 1988; Armes *et al.*, 1989; Akey *et al.*, 1991; Corbett *et al.*, 1996). However, researchers have found some drawbacks associated with using trace elements as insect markers. First, trace elements are not retained well in or on some insect species (Fleischer *et al.*, 1986). Second, high concentrations of trace elements can adversely affect the development of certain insects (Stimmann *et al.*, 1973; Van Steenwyk *et al.*, 1978). Finally, the detection of trace elements through atomic absorption spectrophotometry requires specialized and costly equipment, technical expertise and extensive sample preparation (Akey *et al.* 1991; Hagler & Jackson, 2001).

Recently, we developed a marking procedure that circumvents some of the drawbacks associated with elemental marking. We used the vertebrate-specific proteins, rabbit IgG and chicken IgG, to mark insects (Hagler *et al.*, 1992; Hagler, 1997a). In turn, the protein-marked insects were examined for the presence of the protein by enzyme-linked immunosorbent assay (ELISA) using the corresponding protein-specific antibodies, anti-rabbit IgG and anti-chicken IgG. Applying the proteins onto insects is as straightforward as any of the currently used marking techniques and the analysis of the insects by ELISA is simple and inexpensive. Recently, we showed in laboratory tests that protein has potential use for marking minute hymenopteran parasitoids. For example, we fed individual *Anaphes iole* Girault and *Trichogrammatoidea bactrae* Nagaraja a honey solution containing rabbit IgG. Over 97% of the individuals that fed on the honey containing rabbit IgG remained marked throughout their adult lifespan under laboratory conditions. Furthermore, we showed that these parasitoid species could also be marked externally for their entire adult lifespan with a topical spray of rabbit IgG using various misting and fogging devices (Hagler, 1997b; Hagler & Jackson, 1998).

The present study is the second part of a two part series describing novel techniques to study parasitoid dispersal using MRR techniques. In the first paper, we described how to build a portable suction trap for collecting small parasitoids (Hagler *et al.*, 2002). In this study we examine the short-term retention of various concentrations of rabbit IgG on

Eretmocerus sp. (Ethiopia, M96076), an exotic parasitoid of the silverleaf whitefly, *Bemisia argentifolii* Bellows & Perring [*B. tabaci* (Gennadius), strain B]. We then use these techniques to mark and recapture *E. emiratus* Zolnerowich & Rose (Zolnerowich & Rose, 1998) for field studies designed to investigate its dispersal characteristics. We selected *E. emiratus* for this investigation because: (1) it is one of the few whitefly parasitoid species mass-reared in the quantities needed for conducting a meaningful MRR study; and (2) it is one of the few exotic parasitoids identified as a possible biological control agent of *B. argentifolii* in the southwestern USA (Goolsby *et al.*, 1996, Goolsby *et al.*, 1998; Gould, 1998; Hoelmer, 1998). This is the first open-field study using a protein to mark insects. We believe the techniques applied here will be useful tools for researchers who use MRR techniques.

MATERIALS AND METHODS

Protein Retention Study

Parasitoid marking procedure. The United States Department of Agriculture, Animal and Plant Health Inspection Service, Biological Control Rearing Facility located in Brawley, CA, provided the parasitoids used for this IgG protein marker retention study. Whitefly nymphs parasitized by *Eretmocerus* sp. (Ethiopia, M96076 [The *Eretmocerus* sp. used in this study were originally collected in Ethiopia by G. Terefe and D. Gerling and screened by J. Goolsby at the USDA-APHIS, Mission Biological Control Center, Mission, TX. USA. The #M96076 indicates it is the 76th accession of 1996 for the Mission Biological Control Center quarantine]) were delivered to us 24 h prior to their emergence from their whitefly hosts. Emerging parasitoids were placed into a 2.5 l Tupperware® container and held at 27°C. The container's lid had a 6-cm diameter hole covered with organdy fabric to facilitate air exchange. The parasitoids were provided a food source by streaking several thin lines of honey ($\approx$25 μl) across the lid of the container. After all of the parasitoids had emerged from their hosts they were divided into four groups, each consisting of $\approx$250 individuals. Each group was externally marked with a different concentration of reagent grade rabbit IgG (Sigma Chemical Company, St Louis, MO, USA; #I-5006) using a medical nebulizer (Sunrise Medical, Somerset, PA, USA; Model #800D). A nebulizer, which is a common medical device used to deliver inhaled medications, produces a very fine, fog-like mist (Hagler, 1997b). Briefly, 2.0 mL of a water solution containing 10, 20, 40 or 80 mg of rabbit IgG was placed into the nebulizer. The hose of the nebulizer was then attached to a standard laboratory air outlet and the mouth of the nebulizer was inserted into a 2.5 cm hole (just slightly larger than the mouth of the nebulizer) that was punched out of the side of the Tupperware container. The air outlet was turned on and the parasitoids were 'fogged' until there was no more rabbit IgG solution remaining in the nebulizer ($\approx$2 min). The nebulizer was removed from the container and the 2.5 cm hole in the Tupperware was plugged with a cork. The parasitoids were held in the container for 1 h after fogging and then placed into clean containers containing honey. The protein-marked parasitoids were held in an environmental chamber at 27°C:23°C (L:D), 40% relative humidity (RH), and 14:10 h (L:D). Parasitoids ($n = 7$–28) were removed daily for 2 days after marking and frozen at -70°C until they could be analyzed. Parasitoids serving as negative controls were fed a honey solution for 24 h after emergence. Individual parasitoids were homogenized in 250 μL of tris buffered saline (TBS) at pH 7.4 and assayed for the presence of rabbit IgG by the sandwich ELISA procedure described by Hagler (1997a,b).

Data summary. Mean ($\pm$SD) ELISA absorbance values were calculated for the negative controls. The marked parasitoids were scored positive for rabbit IgG if their ELISA reading was three standard deviations above that of the negative control mean (Hagler 1997a,b). The mean ($\pm$SD) ELISA absorbance values and the percentages of marked parasitoids

scoring positive for rabbit IgG were then tallied for each of the protein concentrations tested over the 2 day retention interval.

Mark-Release-Recapture Study

Study site. The dispersal study was conducted at a 1.4 ha field located at the USDA-ARS, Western Cotton Research Laboratory, Phoenix, AZ, USA on three separate release dates over the summer of 1997. This field has a history of high populations of *B. argentifolii* and the presence of *Eretmocerus eremicus* Rose & Zolnerowich, a native parasitoid species. Several other exotic *Eretmocerus* species that are difficult to distinguish from *E. emiratus* had also been released by one of the authors (JRG) at the study site over the previous four years. Some of the *Eretmocerus* species previously released included *E. emiratus*, *E. mundus* Mercet (from Spain), and *E. hayati* Zolnerowich & Rose (from Pakistan) (Gould *et al.*, 1998). The study site consisted of a plot of cotton, *Gossypium hirsutum* L. 'Delta Pine 5415' that was planted between a small plot of okra, *Hibiscus esculentus* L. 'Clemson Spineless' and a small plot of cantaloupe, *Cucumis melo* L. 'Hales Best Jumbo' (Figure 1). The study site was bordered to the east and west by cotton while the north and south sides of the field were fallow. No pesticides were applied to the field prior to or during the tests. Meteorological data were recorded by a weather station (Campbell Scientific Inc., Logan, UT, USA) located near the experimental plots, providing hourly (averaged 1 min readings) readings of temperature (°C), RH (%), wind speed (m s^{-1}), and wind direction.

Whitefly sampling. A single leaf was selected from the 5th nodal position below the mainstem terminal from 20 randomly selected plants from each of the three crops on the day following each parasitoid release. The leaf disk method described by Naranjo and Flint (1994) was then used to estimate the density of *B. argentifolii* (*Eretmocerus* hosts) nymphs

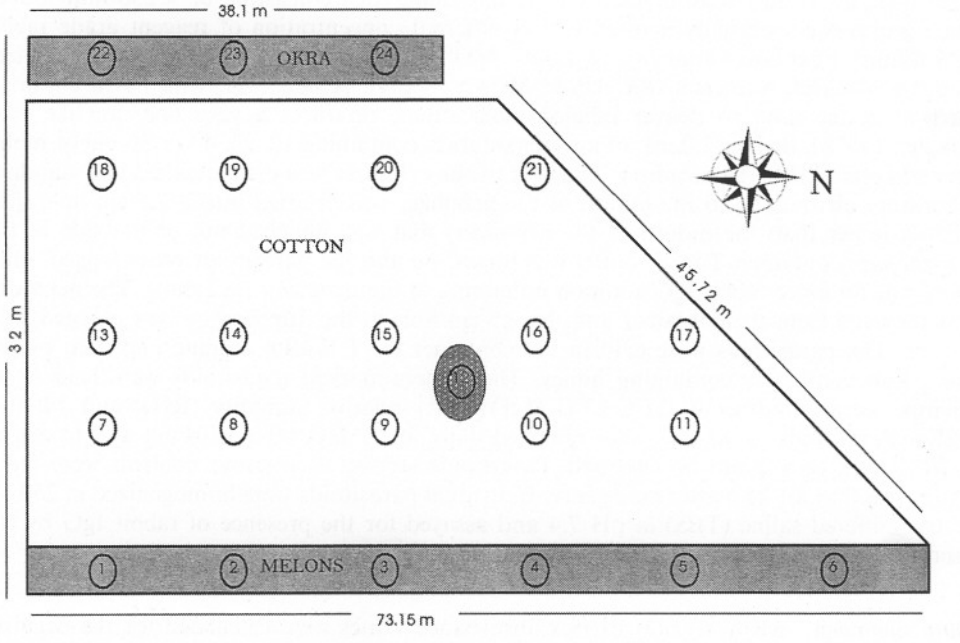

FIGURE 1. Plot design and parasitoid trap placement for the *Eretmocerus emiratus* mark-release-recapture study. The circled numbers are suction trap locations and the shaded circle region around trap 12 is the location of the central point release site.

present on each leaf disk. It should be noted that the cantaloupe plants were in good condition for the first release trial. However, shortly after the first trial the cantaloupe plants began to senesce. By the third release trial the cantaloupe plants were desiccated.

Parasitoid marking procedure. The University of Arizona's Beneficial Insect Rearing Facility located at Tucson, AZ, USA provided parasitoids used for the MRR study. Whitefly nymphs parasitized by *Eretmocerus* sp. (Ethiopia, M96076) (see Zolnerowich & Rose, 1998) were delivered within 24 h prior to their emergence and placed in Tupperware containers as described above. The parasitoids were then dual marked by feeding and fogging. First, the emerging parasitoids were provided with a honey solution consisting of 5.0 mg of rabbit IgG per mL of honey. Several thin lines ($\approx$25 μl) of the protein-enriched honey were streaked across the lid of the container. The parasitoids were kept in the container until they had completely emerged from their hosts ($\approx$48 h). The emerged parasitoids were then marked with 2.0 mL of a 5.0 mg per mL of rabbit IgG solution using the nebulizer described above. The parasitoids were held in the container for 1 h and then released into the center of the cotton plot (Figure 1).

Parasitoid release and recapture. Twenty-four portable suction traps described by Hagler *et al.* (2002) were placed in the field 5.0 m apart down the rows and 3.0 m apart across the rows (Figure 1). Traps were placed in the upper one-third of the cotton and okra canopies ($\approx$1.0 m above the ground) and $\approx$0.1 m above the ground within the cantaloupe canopy. Approximately 117, 38 and 27 thousand protein-marked *E. emiratus* at approximately a 1:1 sex ratio were released at the center of the cotton plot at dusk on 23 July, 4 August, and 13 August 1997, respectively. The suction traps were turned on at 0400 h the day following each parasitoid release. Each trap operated continuously for 32 h. Every 2 h the collection vial was removed from each trap and replaced with a new one. The contents of each trap was labeled indicating its origin and time of day and frozen at -70°C. The number of *Eretmocerus* spp. captured in each trap was tallied, the gender of each individual was determined, and then every individual was assayed for the presence of rabbit IgG by the sandwich ELISA described by Hagler (1997a,b) to determine if they were released parasitoids. Mean ($\pm$SD) ELISA absorbance values were calculated for unmarked negative control parasitoids ($n = 8$ per ELISA plate). Field-collected parasitoids were scored positive for rabbit IgG if their ELISA absorbance value was three standard deviations above the negative control parasitoid mean ELISA value. Field-collected parasitoids that scored negative by the ELISA were assumed to have been present in the field prior to the release of the marked parasitoids (e.g. various *Eretmocerus* species).

Data summary. Daily activity of both marked *Eretmocerus* sp. (Ethiopia, M96076) and unmarked *Eretmocerus* spp. were graphically examined as the total number of parasitoids collected in all 24 suction traps during each 2 h sampling interval. These data were then separated by gender and whether parasitoids contained the protein mark. Finally, the natural distribution of parasitoids (unmarked parasitoids) and the dispersal activity of released (marked) parasitoids was graphically examined using bubble charts in relation to the suction trap locations (Figure 1). Total number of parasitoids collected in each trap was tallied over the entire duration of each MRR trial.

RESULTS AND DISCUSSION

Protein Retention Study

All of the negative control parasitoids examined yielded ELISA absorbance values (0.067 ± 0.007) similar to the TBS blanks (Figure 2). Furthermore, the low ELISA values yielded by unmarked *Eretmocerus* sp. (Ethiopia, M96076) were similar to the ELISA values yielded by other unmarked insect species that have been tested for cross reactivity against anti-rabbit

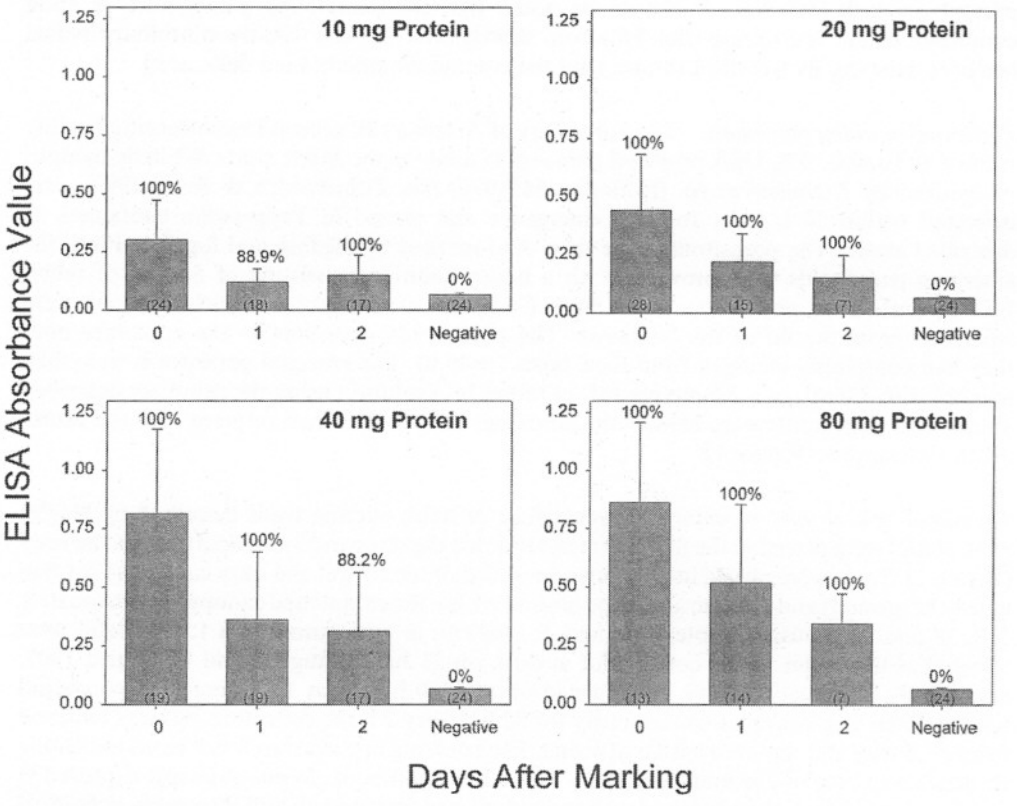

FIGURE 2. Mean ± SD ELISA optical density values (vertical gray bars) and percentage of adult *Eretmocerus* nr. *emiratus* (Ethiopia, M96076) scoring positive (percentage is above the error bars for each treatment) for the presence of rabbit IgG protein after exposure to a topical application of rabbit IgG solution. The number in parenthesis inside each vertical bar is the sample size for each treatment.

IgG (Hagler *et al.*, 1992; Hagler, 1997a,b; Hagler & Jackson, 1998; DeGrandi-Hoffman & Hagler, 2000; Hagler & Miller, 2002). These results indicate that *Eretmocerus* sp. (Ethiopia, M96076) do not contain any proteins that cross react with the anti-rabbit IgG.

Generally, the intensity of the ELISA reaction increased as the concentration of protein applied to the parasitoids increased (Figure 2). For example, the mean ELISA absorbance values yielded for the fogged-marked parasitoids at day 0 were 0.30, 0.44, 0.81 and 0.86 for the 10, 20, 40 and 80 mg protein treatments, respectively. Additionally, the ELISA responses tended to decline for the marked insects as the holding time after marking increased for each of the protein concentrations. Parasitoids marked with 80 mg of protein yielded mean ELISA values of 0.86, 0.52 and 0.34 at 0, 1 and 2 days, respectively, after marking.

The lack of reactivity by the unmarked parasitoids combined with the minimal variation between the samples are important characteristics of the protein marking technique. The positive ELISA threshold value for *Eretmocerus* sp. (Ethiopia, M96076) (i.e. the mean + 3 SD of the unmarked parasitoids) was only 0.088. Regardless of the amount of protein applied or the post-marking interval, 98% of the marked parasitoids contained enough rabbit IgG to be easily distinguished from unmarked parasitoids (Figure 2). These results indicate that any of these protein concentrations would be sufficient for marking *Eretmocerus* spp. for at least 2 days. However, the rapid decay of the ELISA response for the parasitoids marked

with the lower concentrations of rabbit IgG (e.g. 10 and 20 mg) suggest that more concentrated protein solutions should be used for MRR studies lasting more than 2 days. We have subsequently been marking parasitoids for MRR studies lasting 4 days with 40 to 100 mg of rabbit IgG (JRH, unpubl. data).

Mark-Release-Recapture Study

There is extensive laboratory or greenhouse research dedicated to investigating the behavioral and dispersal characteristics of whitefly parasitoids (Gerling, 1966a–c; Gerling & Fried, 1997; Headrick *et al.*, 1995, 1997; Hoddle *et al.*, 1997a,b, 1998a,b; Heinz, 1995; Heinz & Parrella, 1998, many others). Unfortunately, there is little information on the behavioral and dispersal characteristics of whitefly parasitoids in open-field situations (Simmons, 2000; Bellamy & Byrne, 2001). A major reason for this lack of information is that whitefly parasitoids are difficult to track in nature because they are small ($\approx$0.5 mm long) and elusive. The battery-operated suction traps (Hagler *et al.*, 2002) and the protein marking procedure used in this study were useful tools for studying the dispersal characteristics of *Eretmocerus* sp. (Ethiopia, M96076). The traps are ideal for open-field MRR studies that require grid-like sampling schemes because the traps are inexpensive, lightweight, durable, easy to operate, require minimal handling of the samples, selective for non-destructive capture of tiny insects, and run continuously for extended periods of time (see Hagler *et al.*, 2002). These characteristics minimize the time in sorting, identification and analysis.

The goal of this study was to describe and validate new techniques for open-field MRR studies. In the process, we discovered many interesting characteristics about *E. emiratus* dispersal behavior. A total of 1388, 637 and 397 parasitoids (marked and unmarked) were captured during the first, second, and third MRR trials, respectively. A large proportion (42.5%) of the parasitoids were captured between 0600 and 1000 h with the peak activity occurring between 0600 and 0800 h (Figure 3). Virtually no parasitoids were captured at night and very few were captured during the afternoon when ambient summer temperatures in Arizona usually exceed 40°C. Whether *Eretmocerus* sp. (Ethiopia, M96076) activity varies in different regions of the world or during the cooler seasons in Arizona are areas for future investigation.

The protein marking procedure and the ELISA provided us with a precise method to distinguish released parasitoids from their naturally occurring counterparts. Although the sex ratio of the released parasitoids was $\approx$1:1, our traps consistently captured 7 to 9 times more males than females (Figure 4). Furthermore, the proportion of males captured did not differ whether they were marked or unmarked. For example, the percentages of recaptured marked parasitoids that were male were 91.2, 79.5 and 85.1% and the percentages of unmarked captured parasitoids that were male were 89.4, 73.4 and 85.7% for MRR trials 1, 2, and 3, respectively (Figure 4). The reason(s) why we captured more males than females is currently under investigation at our laboratory.

A total of 1499 unmarked parasitoids were collected during all three trials (Figure 5(a)–(c)). The majority, 81.0%, of unmarked parasitoids were collected in the cantaloupe plot during the first release trial. On average, 67.4 ± 98.4, 5.6 ± 8.8 and 3.0 ± 2.6 unmarked parasitoids were collected per trap in the cantaloupe, cotton and okra plots, respectively. The greater distribution of naturally occurring (unmarked) *Eretmocerus* in the cantaloupe plot during the first and second MRR trials was undoubtedly due to the heavy density of whitefly hosts in the cantaloupe plot (Table 1). However, even though there were plenty of whitefly hosts still present in the cantaloupe during the third trial, not many unmarked parasitoids were captured in these traps. This was probably due to the rapid senescence of the cantaloupe plants and associated decline of parasitoid hosts (Table 1). Legaspi *et al.* (1997) also suggested that a contributing factor to whitefly parasitoid migration from cotton was due to a decline in the quality of the cotton plant.

Almost 40% ($n = 930$) of the 2429 captured parasitoids were positively identified for the presence of the protein marker (Figure 6(a)–(c)). On average, 17.0 ± 30.3, 7.7 ± 9.0, and

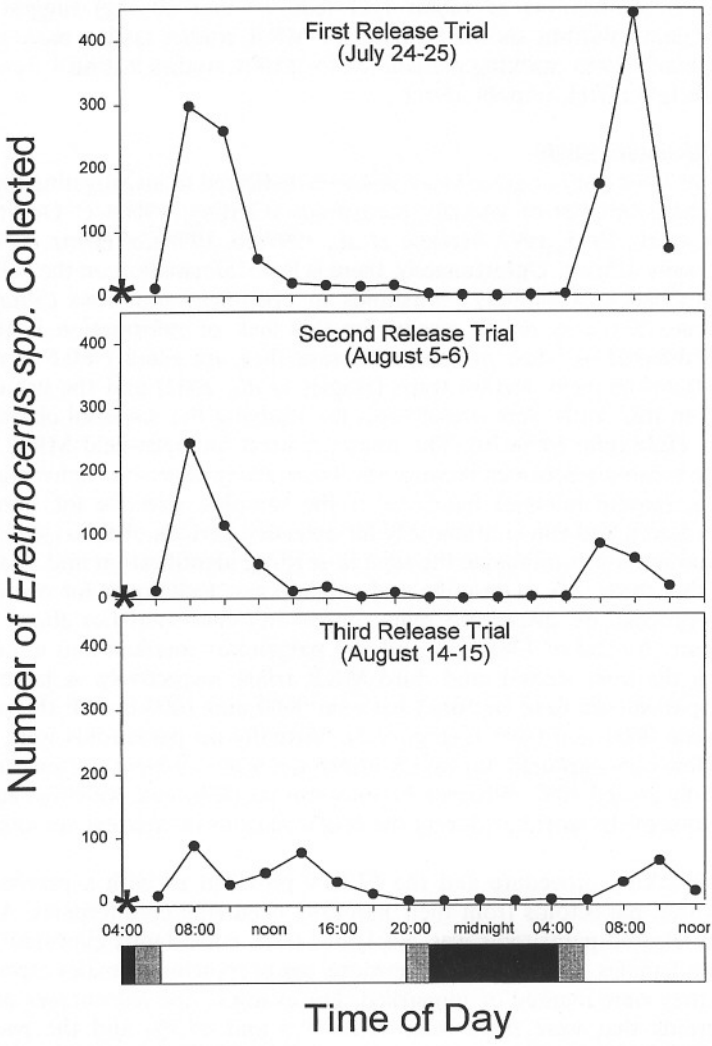

FIGURE 3. Total number of *Eretmocerus emiratus* collected from the 24 suction traps over each 2-h sampling interval. The asterisk indicates the time that the suction traps were started. A summed total of 1388, 637 and 397 *E. emiratus* were collected during the first, second, and third release trials, respectively. The bar along the *x*-axis indicates total darkness (black), dawn and dusk (gray) and bright sunlight (white).

2.1 ± 1.5 marked parasitoids were captured per trap in the cotton, cantaloupe and okra plots, respectively. The majority of the parasitoids captured in the cotton plot were captured at or adjacent to the release site (e.g. in traps 9, 10, 12, 15 and 16, see Figures 1 and 6) in the cotton plot. Interestingly, as the quality of the cantaloupe plants declined with each trial, the distribution of the parasitoids around the central point release site in the cotton became more evenly distributed. For example, 86.2, 61.1 and 51.9% of the recaptured parasitoids were captured east of the central point release site during the first, second and third MRR trial, respectively.

The tendency for parasitoid movement to the east during the first two trials could be due to several factors. First, weather conditions might influence the direction that the parasitoids

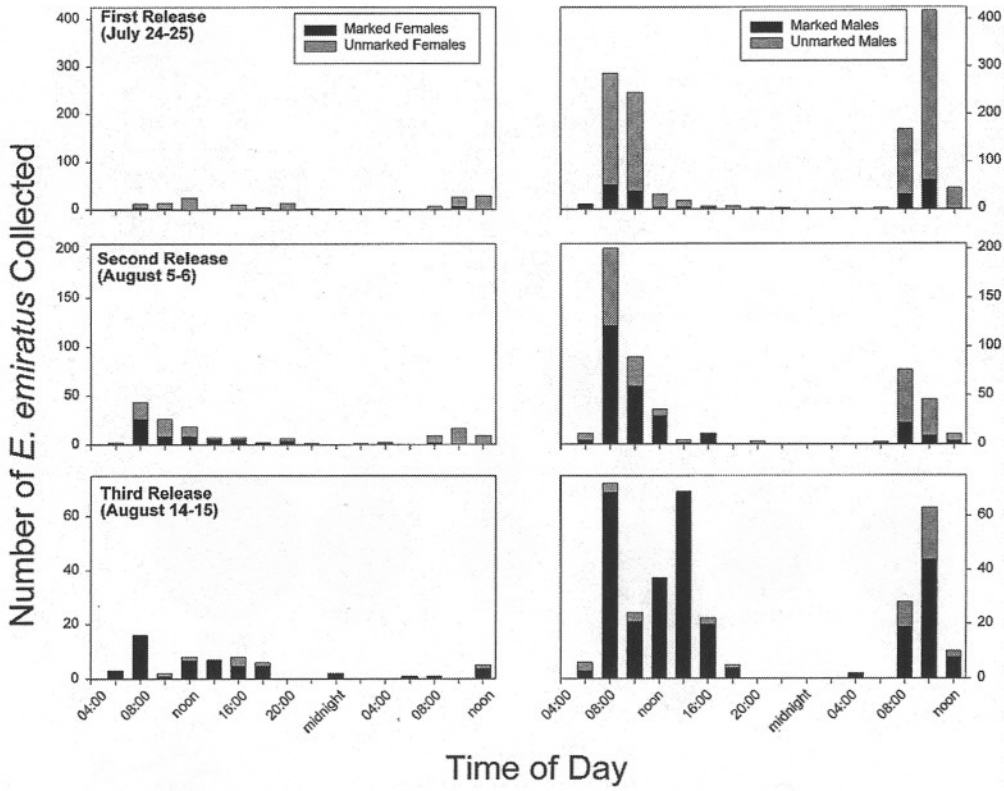

FIGURE 4. Stacked bar charts showing the total number of marked and unmarked female (left) and male (right) *Eretmocerus emiratus* collected during each 2 h sampling interval.

move from the central point release site. Abiotic factors such as temperature, wind speed and wind direction (upwind and downwind) are frequently cited as major factors influencing insect movement (Hendricks, 1967; Biever, 1972; Messing *et al.*, 1995; Corbett & Rosenheim, 1996; Fournier & Boivin, 2000; many others). The average temperature, wind speed and RH were similar during each trial. Overall, the wind speed was relatively slow and there were no prevailing winds, so the extent to which wind aided or hindered parasitoid dispersal could not be determined. Second, parasitoid movement could be influenced by the cropping pattern. As shown in Figure 5, the natural density of *Eretmocerus* spp. was the greatest in the cantaloupe plot. Furthermore, marked parasitoids were more frequently captured in the traps located in the cantaloupe plot than in the traps located similar distances away from the central point release site in the cotton and okra plots during the first two MRR trials (Figure 6(a)–(C)). It is well documented that cantaloupe is a preferred whitefly host (Coudriet *et al.*, 1985; Chu *et al.*, 1995). Not surprisingly, the number of potential whitefly hosts was much higher in the cantaloupe than in the cotton or okra (Table 1). It seems plausible that the released parasitoids were moving toward the east in response to either visual or volatile cues emitted by whiteflies or cantaloupe (Guerrieri, 1997). Heinz and Parrella (1998) conducted a study of the host-finding capabilities of five *B. argentifolii* parasitoids from different geographical regions. They found a high degree of variation in the olfactory responses of the various parasitoid species to odors emitted from whitefly-infested foliage. More studies investigating various host-finding cues used by whitefly parasitoids are sorely needed. Third, parasitoid movement could be due to a positive

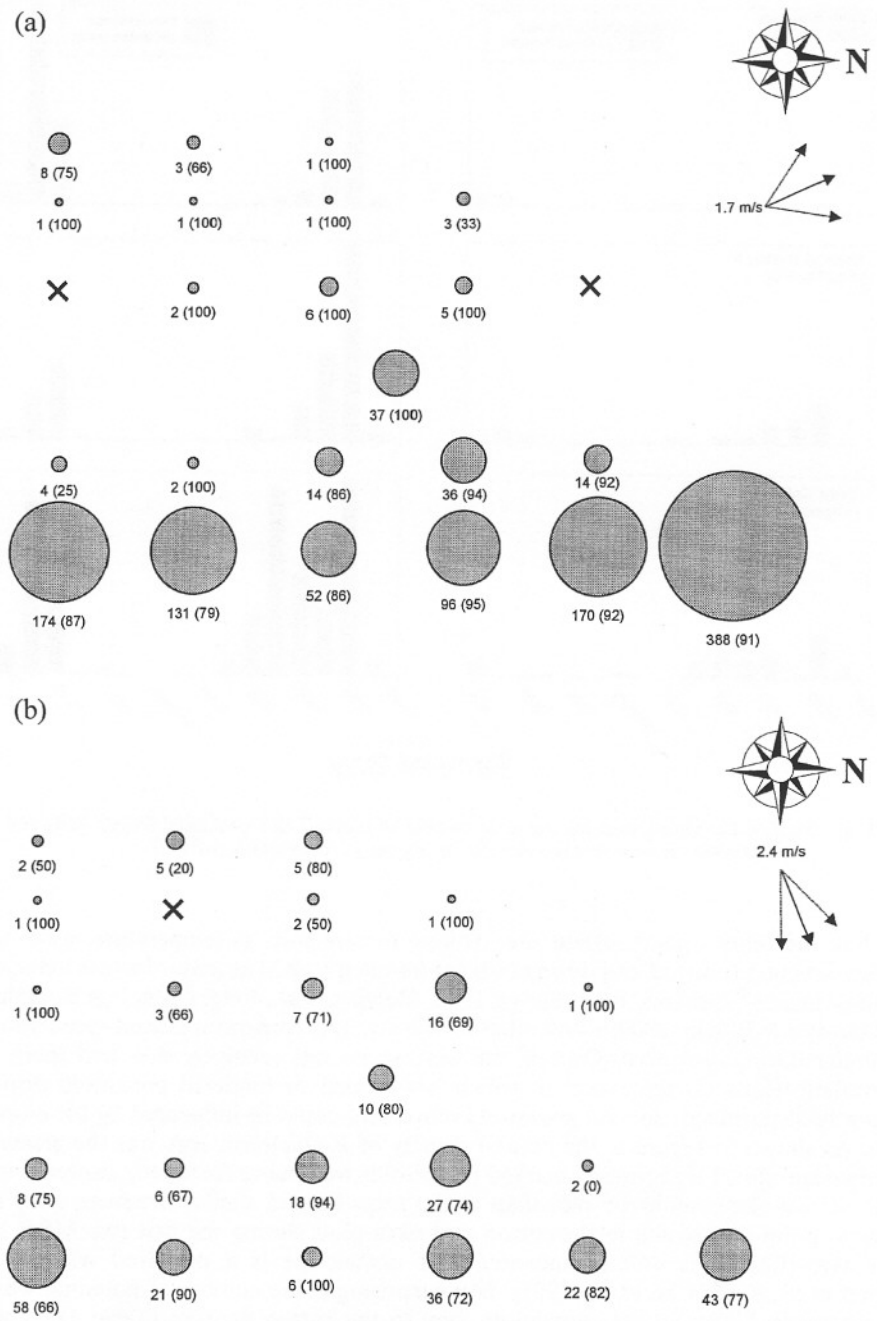

FIGURE 5. Bubble graphs indicating the natural distribution of *Eretmocerus* spp. during the first (a), second (b) and third (c) mark-release-recapture trial. The numbers below each bubble are the total number of unmarked *E. emiratus* collected from each suction trap (see Figure 1 for a map of the release site) over each 32-h sampling period. The numbers in parentheses below each bubble is the percentage of males collected in the traps. An '×' indicates those trap locations where no parasitoids were captured. The solid arrow and the dotted arrows are the mean and standard deviation, respectively of the prevailing wind direction during each trial. The number next to the arrow is the average wind speed.

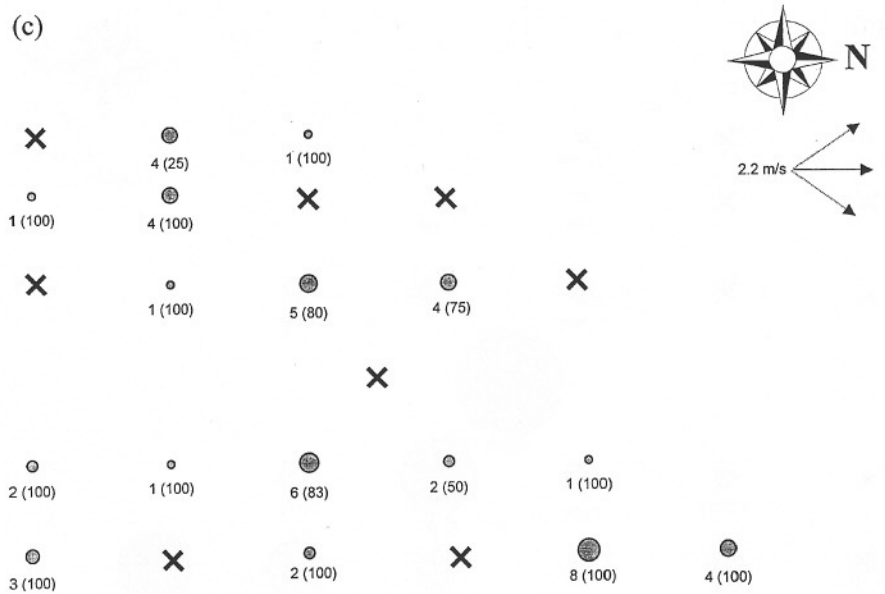

FIGURE 5. *Continued.*

TABLE 1. The mean ± SD number of *Bemisia argentifolii* nymphs on 3.88 cm^2 leaf disks taken from cotton, cantaloupe, and okra leaves during the three *Eretmocerus* sp. (Ethiopia, M96076) mark-release-recapture trials in Phoenix, AZ, USA, 1997

	Mean ± SD *B. argentifolii* nymphs/leaf disk		
MRR Trial	Cotton	Cantaloupe	Okra
1	0.55 ± 0.88	79.15 ± 72.03	2.90 ± 2.84
2	0.05 ± 0.22	248.00 ± 256.67	0.70 ± 0.73
3	0.01 ± 0.01	81.90 ± 130.94	0.55 ± 0.82

phototactic response to sunlight exhibited by *Eretmocerus* sp. (Ethiopia, M96076). Clearly *Eretmocerus* spp. (JRH, pers. obs.) and other insect species exhibit positive phototactic responses to light (Muirhead-Thomson, 1991). Our field studies demonstrated that parasitoid activity is greatest shortly after sunrise (Figure 3). Perhaps this parasitoid species is moving toward the sunlight (east) during the period in which they are most active. We have found in subsequent studies that *Eretmocerus* sp. (Ethiopia, M96076) consistently aggregate toward the east end of enclosed field cages during early morning hours (JRH, unpubl. data). Finally, it is probable that a combination of these factors influence the movement of *Eretmocerus* sp. (Ethiopia, M96076). Studies on the influence of whitefly host density, habitat structure (e.g. host plant, cropping sequence, plant architecture etc.) and abiotic forces (e.g. photoperiod, wind speed, wind direction etc.) on *Eretmocerus* spp. movement are currently underway at our laboratory.

In summary, this is the first study that used the protein marking technique and the suction trap for an open-field MRR study. Moreover, this is the first study that we are aware of documenting the open-field dispersal characteristics of the exotic whitefly parasitoid,

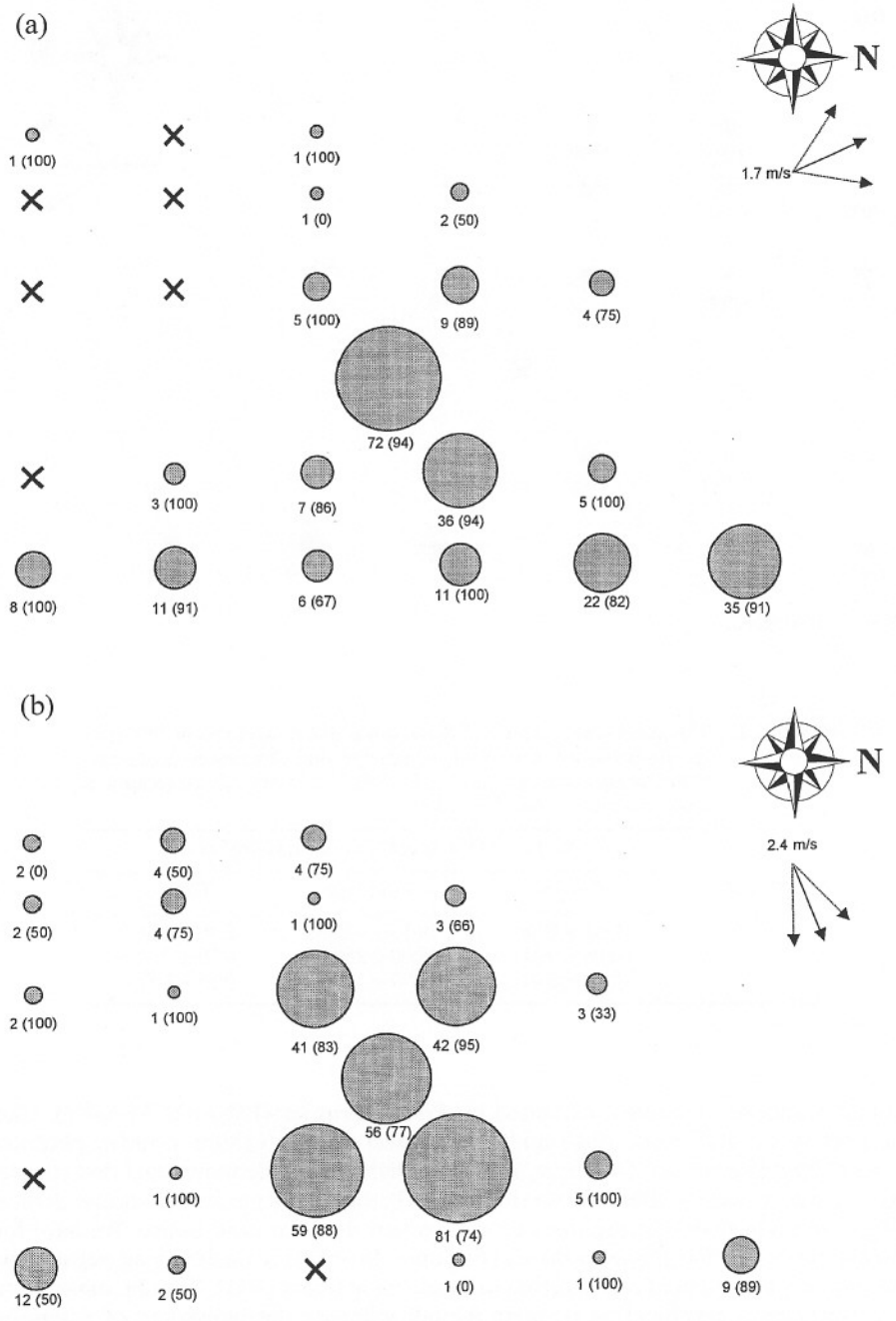

FIGURE 6. Bubble graphs indicating the dispersal pattern of marked and released *Eretmocerus emiratus* during the first (a), second (b) and third (c) MRR trial. The numbers below each bubble is the total number of marked *E. emiratus* collected from each trap (see Figure 1 for a map of the release site) over each 32-h sampling period. The numbers in parentheses below each bubble is the percentage of males collected in the traps. An '×' indicates those trap locations where no parasitoids were captured. The solid arrow and the dotted arrows are the mean and standard deviation, respectively of the prevailing wind direction during each trial. The number next to the arrow is the average wind speed.

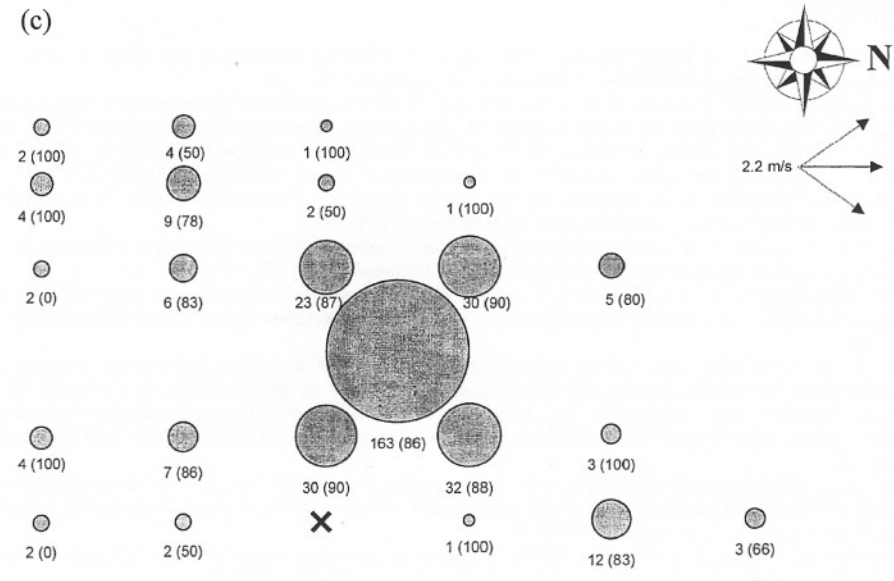

FIGURE 6. *Continued.*

Eretmocerus sp. (Ethiopia, M96076). The suction trap described in detail by Hagler *et al.* (2002) proved to be ideal for parasitoid MRR studies. The protein marker coupled with the protein-specific ELISA proved to be effective methods for labeling minute parasitoids to distinguish them from naturally occurring ones. Using these methodologies, we showed that *Eretmocerus* sp. (Ethiopia, M96076) disperse most actively shortly after sunrise. Furthermore, it appears that there are gender differences in their dispersal characteristics. Many parasitoids were collected early each morning east of the central point release site during two of the three MRR trials. Further studies are underway to identify the effect of cropping sequence and abiotic factors (e.g. photoperiod, wind direction, wind speed, temperature, humidity etc.) on the movement of various whitefly parasitoids. Additionally, studies are underway to examine gender-specific dispersal behaviors. It is critical that we determine the fate of female parasitoids released in augmentative biological control programs in order to increase the probablity of effective pest control.

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